Reference: *Biol. Bull.* **174**: 54–66. (February, 1988)

A Physiological Comparison of Bivalve Mollusc Cerebro-visceral Connectives With and Without Neurohemoglobin. I. Ultrastructural and Electrophysiological Characteristics

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Abstract. The presence of hemoglobin in tissues of a small number of species is puzzling when homologous tissues of closely related species do not possess hemoglobin. Several species of bivalve molluscs possess nervous systems with nerve hemoglobin (neurohemoglobin). These systems were compared to nervous systems from other bivalve molluscs without neurohemoglobin to determine ultrastructural and electrophysiological characteristics under normoxic conditions in an attempt to locate any differences between these two types of nervous systems. Cerebro-visceral connectives from the bivalves *Tellina alternata* and *Spisula solidissima* with neurohemoglobin and *Tagelus plebeius* and *Geukensia demissa* without neurohemoglobin possess a perineural sheath, a subjacent peripheral layer of glial cells, and glial cell processes that enwrap bundles of 0.2–0.4 μm diameter axons. Neurohemoglobin-containing cerebro-visceral connectives have smaller axon bundles and more dense perineural sheaths than those without neurohemoglobin. These features may be important in oxygen delivery from the neurohemoglobin to the axons. Action potential traces, conduction velocities, refractory periods, strength-duration relationships, and temperature responses of all four connectives are typical of nerves possessing very small axons. There are no obvious electrophysiological differences between cerebro-visceral connectives with and without neurohemoglobin.

Introduction

Tissue hemoglobins are distributed sporadically throughout most of the biological taxa. In general, hemoglobins may participate in the facilitated diffusion of oxygen or they may serve to store oxygen for use during periods of low oxygen availability (Colacino and Kraus, 1984; Burr and Harosi, 1984; Kraus and Colacino, 1986). Because hemoglobin can occur in a particular tissue of one or a few species of animals and not in homologous tissues of the majority of closely related species, the question arises as to why the need for hemoglobin exists. If hemoglobin is needed to augment the oxygen supply to a specific tissue, why are the oxygen demands of the homologous tissues with and without hemoglobin different? What, if anything, is different about the structure, physiology, or biochemistry of each tissue?

The hemoglobin-containing nerves of marine invertebrates represent an ideal system for this type of study for several reasons. First, although the nervous systems of several organisms tolerant of anoxic conditions can respond to stimulation in the absence of oxygen (Mangum, 1980; Surlykke, 1983), nervous tissue in general is functionally dependent on aerobic metabolism and thus on oxygen availability. In addition, nervous function can be readily monitored. Second, nerve hemoglobins have been noted in many invertebrates from several phyla, but it is by no means common (Wittenberg *et al.*, 1965). Therefore, it is possible to locate species without nerve hemoglobins which are related to species with nerve hemoglobins for comparative study.

The connectives between ganglia in the bivalve molluscs are relatively simple in structure and function. They are distinct anatomical structures, self-contained within a protective sheath, and are thus easily prepared for ultrastructural and electrophysiological study. Moreover, tissue function can be monitored unambiguously

Received 20 May 1987; accepted 12 November 1987.

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because the axons are relatively homogeneous in type and size. The cerebro-visceral connectives, which are the main routes for communication between the cerebropleural and visceroparietal ganglia, are relatively long in bivalves with an elongated anteroposterior axis and thus were selected for this work.

Cerebro-visceral connectives from several bivalve molluscs lacking hemoglobin have been examined with respect to ionic and osmotic stresses (Willmer, 1978a, b), ionic permeability (Satelle and Howes, 1975), and ultrastructural organization (Gupta *et al.*, 1969). Kraus and Colacino (1986) reported that neurohemoglobin in bivalve cerebro-visceral connectives may serve as an oxygen supply for nervous activity during anoxic conditions. In this paper, basic ultrastructural and electrophysiological characteristics of the cerebro-visceral connectives from the bivalves *Tellina alternata* and *Spisula solidissima,* which are bright red with neurohemoglobin, and the white cerebro-visceral connectives of the bivalves *Tagelus plebeius* and *Geukensia demissa,* which lack neurohemoglobin, were determined under normoxic conditions in an attempt to locate any differences that may exist between these two types of connectives. The first three bivalves belong to the same order while *G. demissa* belongs to a separate subclass.

Materials and Methods

Specimens of *Tellina alternata* and *Tagelus plebeius* were collected from the ebb tide level of a backwater sandy mud flat at Long Beach, North Carolina. Specimens of *Geukensia demissa* were collected at the flood tide level from a *Spartina* bed bordering the same mud flat. Specimens of *Spisula solidissima* were collected offshore and supplied by the Rutgers Shellfish Research Laboratory, Port Norris, New Jersey, and the United States Department of Commerce-NOAA, Milford, Connecticut. Most animals were obtained during the spring and summer.

The specimens were maintained in aerated seawater aquaria, 35‰ salinity, at 15–20°C. They were fed weekly with fish food (TetraMin, Carolina Biological) that was homogenized and stirred into the tank. Animals were observed siphoning the suspended food particles and food was found inside the animals' digestive diverticula during dissection. Their activity level and overall condition appeared stable for at least two months. Animals were usually used for experimentation within four weeks of collection. Animal sizes and weights are listed in Table I.

Dissection of cerebro-visceral connectives

Clams were opened by severing the adductor muscles, and held open for dissection with rubber bands in a glass bowl containing cold seawater. The siphons, gills, and palps were removed to expose the visceral and cerebral ganglia. An entire single cerebro-visceral connective was exposed by gently teasing away the embedding body wall musculature, taking great care not to stretch the connective or puncture the sheath. Once exposed, the connective was severed at the base of the ganglia on both ends and removed for experimentation. It was unnecessary to ligature the severed ends because the dense cellular matrix and tough perineural sheath prevented the neural contents from exuding out of the cut ends. One or both of the connectives were used from each animal. Entire connectives ranged from 2–4 cm to 6–8 cm in length and from 0.01 cm to 0.025 cm in diameter (*T. alternata* and *S. solidissima,* respectively). With some practice, the operation could be completed within 30 min.

Light and transmission electron microscopy

The cerebro-visceral connective, at approximately ¼ its length posterior to the cerebral ganglion, is free from branches and lies loosely inside the body wall. Connective segments, 0.5 to 1 cm in length, were excised from this location and fixed immediately in 2.5% glutaraldehyde in 0.2 *M* Millonig's phosphate buffer (pH 7.6) and 0.34 *M* NaCl for 0.5 h. After rinsing twice in Millonig's buffer, the segments were postfixed for 0.5–1.0 h in 2% OsO_4 in Millonig's phosphate buffer (pH 7.6). Following postfixation, specimens were dehydrated through a graded series of ethanols and embedded in Polybed 812 using propylene oxide as the infiltration solvent.

Thin sections were cut with diamond knives on a LKB Ultratome Nova at various positions along the length of the fixed connective segments. Sections were collected on bare copper hexagonal mesh grids, stained with alcoholic uranyl acetate and aqueous lead citrate, and photographed with a Phillips EM 300 transmission electron microscope. Electron micrographs of thin sections (5–10 sections from each of 4–5 individuals of each species) were used to determine the following distances and counts: connective diameter, perineural sheath thickness, axon density, axon size distribution, number of axons per bundle, and the greatest distance between the central axon and nearest glial cell within each bundle.

Electrophysiological measurements

To measure electrophysiological properties, an anterior segment of the cerebro-visceral connective, measuring approximately 2.5 cm in length, was excised and pipetted into a temperature-controlled nerve chamber (Fig. 1). While submerged in the chamber, the connective was positioned to lie across 12 platinum electrodes. The bathing fluid was slowly asperated beneath the electrodes until they suspended the moist connective in air. The chamber floor was covered with moistened lens pa-

Table I

Animal sizes and weights

Parameter	*Tellina alternata*	*Spisula solidissima*	*Tagelus plebeius*[a]	*Geukensia demissa*[a]
Maximum length, cm	5.0 ± 0.3[b]	7.8 ± 0.4	8.4 ± 0.3	10.1 ± 0.3
Fresh tissue weight, g[c]	3.4 ± 0.7	20.8 ± 3.6	14.7 ± 0.8	9.2 ± 1.7
Valve weight, g	5.3 ± 1.2	26.7 ± 3.4	9.8 ± 0.9	29.5 ± 8.9

[a] Species without neurohemoglobin.
[b] Values are given as mean ± standard deviation of 8–12 individuals.
[c] Tissue was removed from valves and blotted dry before weighing.

per to prevent dehydration of the connective and the chamber was sealed with a glass lid. Water did not condense on the connective during experimentation. All experiments were performed under normoxic conditions.

The connective was stimulated externally with a square wave stimulator (Grass SD9). The resultant compound action potential and stimulus pulse were monitored with a preamplifier (Narco) and microcomputer (Apple IIe) equipped with dual trace oscilloscope hardware and software (RC Electronics). Action potential traces were used to make the following measurements: action potential amplitude, conduction velocity, absolute and relative refractory periods, and the strength-duration relationship. Measurements were made at three temperatures after a 30 min equilibration period at each temperature (5, 10, and 15°C for *S. solidissima* and 10, 15, and 20°C for the others). Several electrophysiological parameters were determined with each preparation. Measurements were made with preparations from 5–8 individuals of each species.

Ultrastructural and electrophysiological data were analyzed using hierarchical analysis of variance to determine if variation could be attributed more to differences among individual species or between species with and without neurohemoglobin.

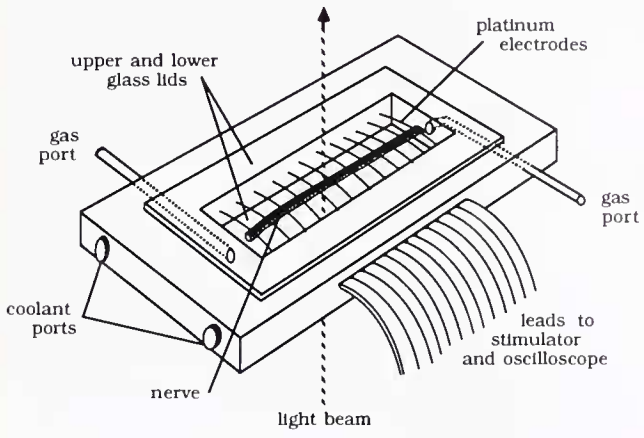

Figure 1. The nerve-chamber gas slide used for electrophysiological measurements.

Results

Neuroanatomy and ultrastructure

The bilaterally symmetrical nervous systems of lamellibranch bivalves typically have three pair of ganglia (visceroparietal, cerebropleural, and pedal), a number of major nerve trunks and connectives, and several finer nerve branches. The entire nervous system is contained within a tough, thin connective tissue-like sheath. The neuroanatomy of all four species of bivalves in this study is typical of lamellibranch bivalves with elongated anteroposterior axes (see Fig. 24.2, Bullock, 1965). However, the nervous systems differ in one regard: those of *Tellina alternata* and *Spisula solidissima* are vivid red while those of *Tagelus plebeius* and *Geukensia demissa* are unpigmented or white. The red color results from the presence of neurohemoglobin in the glial cells of the connectives (Kraus and Colacino, 1986; Doeller and Kraus, 1988).

The general anatomical design is similar among the cerebro-visceral connectives from all species examined. The connectives are surrounded by a 1–3 μm thick, fairly uniform acellular sheath that resembles the neural lamella of other invertebrate nervous systems (Horridge and Bullock, 1965). Situated subjacent to the sheath are glial cell bodies. Processes of glial cells ramify throughout the connectives and ensheath bundles of axons.

Ultrastructurally, the perineural sheaths of connectives with neurohemoglobin possess 20–30 tightly arranged concentric laminae consisting of a thick, electron-dense extracellular matrix material (Fig. 2). The individual laminae appear closer together, thinner, and more complete at the inner margin of the sheath. The outer laminae are about 60–70 nm apart. In contrast, the sheaths of connectives without neurohemoglobin consist of loosely arranged, thin laminae (Fig. 2) and resemble the cerebro-visceral connective sheaths of *Anodonta cygnea* (Gupta *et al.*, 1969) and *Mytilus edulis* (Willmer,

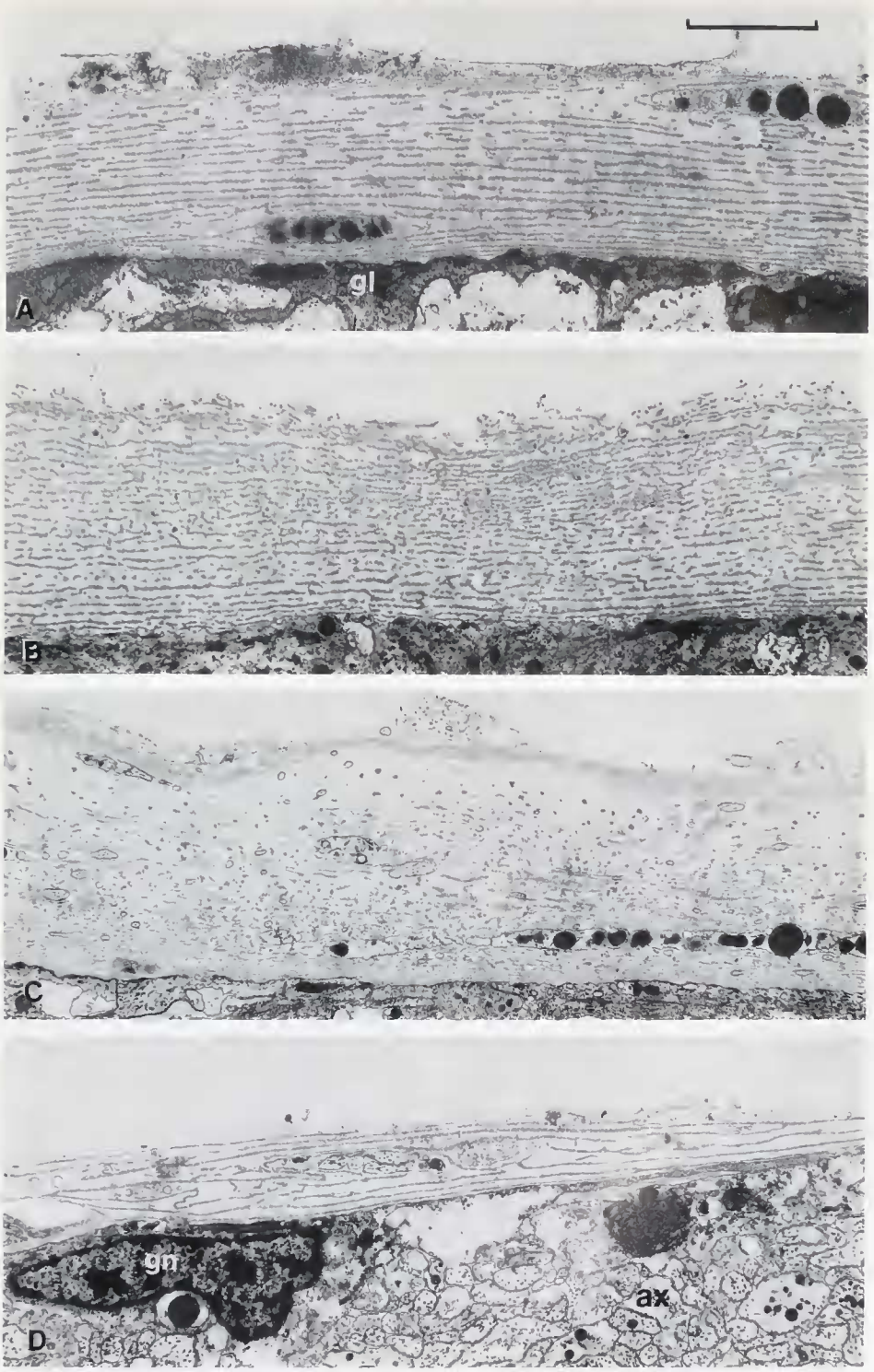

Figure 2. The neural sheaths of bivalve cerebro-visceral connectives. A. *Tellina alternata.* B. *Spisula solidissima.* C. *Tagelus plebeius.* D. *Geukensia demissa.* Bar is 2 μm for A–D. Glial cell, gl; glial cell nucleus, gn; axons, ax.

Table II

Morphometric characteristics of the cerebro-visceral connectives

Parameter	*Tellina alternata*	*Spisula solidissima*	*Tagelus plebeius*[a]	*Geukensia demissa*[a]
Connective diameter, μm	126 ± 21.0[b]	231 ± 9.7	169 ± 10.2	212 ± 35.4
Sheath thickness, μm	2.64 ± 0.74	3.58 ± 1.20	3.12 ± 0.50	2.01 ± 0.61
Axons/bundle[c]	72 ± 32	54 ± 40	177 ± 86	>250[d]
Cross sectional area of axon bundle, μm^2	5.16[e]	9.57	13.1	>36.6[d]
Maximum distance from axon to glial cell, μm	1.23 ± 0.50	1.44 ± 0.84	1.66 ± 0.45	≥6.7[d]
Density axons/μm^2 (axons counted)	6.18 ± 3.61 (7572)	2.12 ± 1.27 (2712)	4.50 ± 0.67 (5305)	3.67 ± 0.84 (4675)
Axons/connective (approximate)	73,000[f]	89,000	101,000	129,000
Volume of axons, % of connective	44.3[g]	37.6	36.2	53.8
Axon membrane, cm^2/g wet weight of connective	56,000[g]	30,000	43,000	47,000

[a] Species without neurohemoglobin.
[b] Unless specified, values are given as mean ± standard deviation of measurements obtained from 5–10 sections from 4–5 individuals.
[c] $P < 0.025$ between connectives with and without neurohemoglobin, using hierarchical ANOVA.
[d] Axon bundles were larger than electron micrographs.
[e] Calculated from axons/bundle and mean axon diameter (Fig. 6).
[f] Calculated from axon density and connective diameter.
[g] Calculated from axons/connective and mean axon diameter.

1978b), both which most likely lack neurohemoglobin. Fibers that run parallel to the long axis of the connectives are scattered between the laminae in all sheaths examined.

General morphometric characteristics are listed in Table II. Connectives with and without neurohemoglobin have similar axon density and relative axon volume. They possess a large axon membrane surface area, similar to the garfish olfactory nerve with 65,000 cm^2/g (Easton, 1971). In addition, there is no significant difference in sheath thickness between cerebro-visceral connectives with and without neurohemoglobin. However, axon fields in connectives with neurohemoglobin are more subdivided by glial cell processes than axon fields in connectives without neurohemoglobin, thus connectives with neurohemoglobin possess significantly fewer axons per bundle (Fig. 3; Table II). Cerebro-visceral connectives without neurohemoglobin from species in this study and from *Mytilus edulis* possess large, densely packed axon fields, relatively uninterrupted by glial cell processes (Fig. 3; Willmer, 1978b). This difference in architecture results in smaller axon bundles and shorter mean diffusion distances between axons and glial cells in neurohemoglobin-containing connectives (Fig. 3; Table II).

Glial cells in red connectives possess a granular, electron-dense cytoplasm (Fig. 3), presumably due to the presence of neurohemoglobin (Ruppert and Travis, 1983; Doeller and Kraus, 1988). Prior and Lipton (1977) also report a granular, electron-dense cytoplasm in the glial cells of *S. solidissima* siphonal nerves. In contrast, the cytoplasm of glial cells in unpigmented connectives appears much less dense (Fig. 3).

In all four cerebro-visceral connectives, more than 75% of the axons fall within one standard deviation of the mean axon diameter for each species (Figs. 4, 5). Although the larger connectives tend to have larger mean axon diameters (Fig. 5), the differences in mean axon diameter cannot be attributed to the presence of neurohemoglobin. The mean cerebro-visceral axon diameter (0.432 μm) of the mussel *G. demissa* (Fig. 5) is very similar to the mean cerebro-visceral axon diameter (0.403 μm) of the mussel *Mytilus edulis* (Willmer, 1978b).

Electrophysiology

Examples of externally recorded compound action potentials from cerebro-visceral connectives at different temperatures are shown in Figure 6. Action potentials conducted in either direction were indistinguishable in a given preparation, thus other electrophysiological characteristics were measured without regard to conduction direction. Action potential traces typically consisted of a stimulus artifact, a few fast, low-amplitude waves, and a

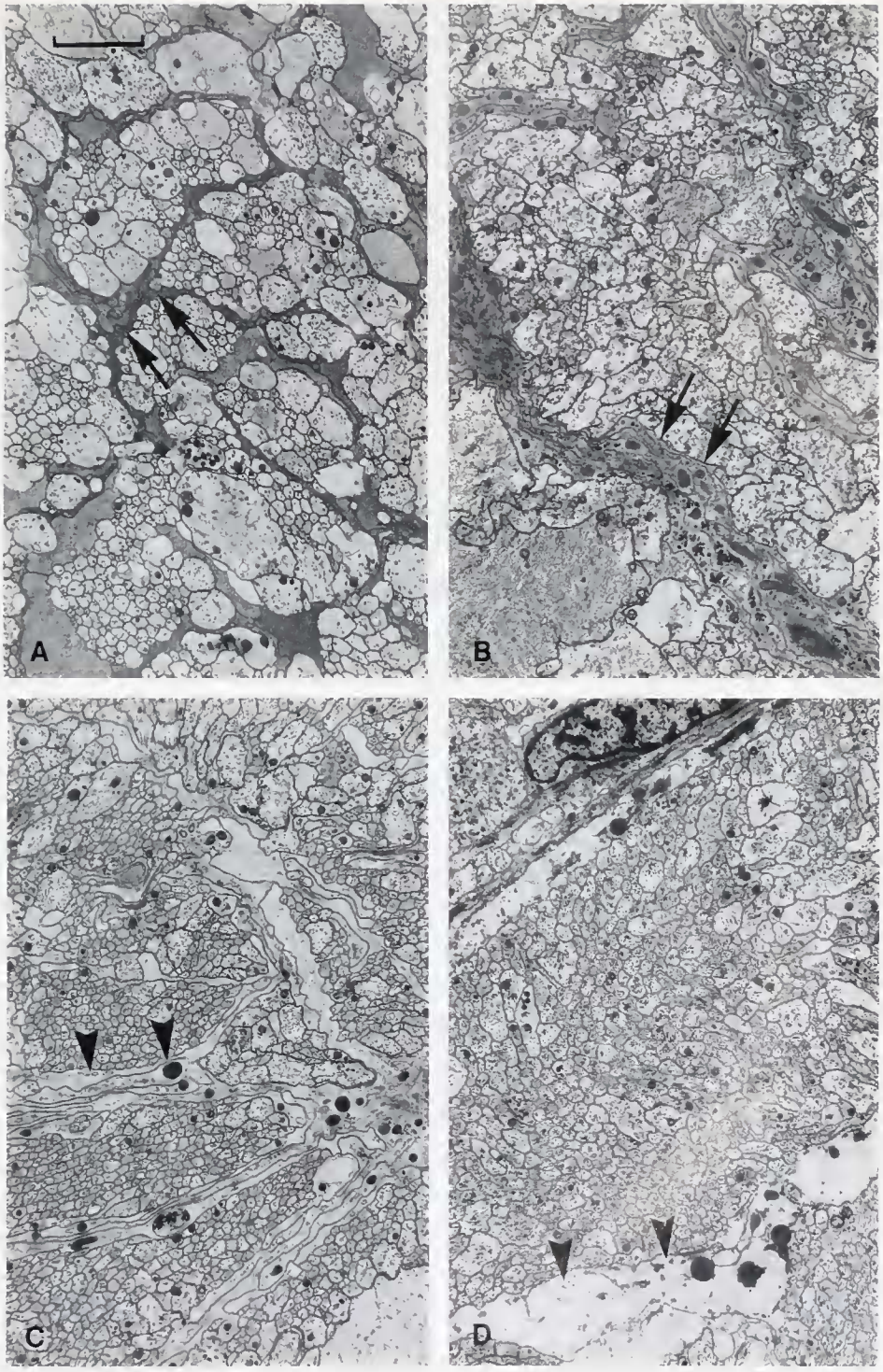

Figure 3. Axon bundles of bivalve cerebro-visceral connectives. A. *Tellina alternata*. B. *Spisula solidissima*. C. *Tagelus plebeius*. D. *Geukensia demissa*. Bar is 2 μm for A–D. Note the neurohemoglobin-containing glial cells (arrows) of *Tellina alternata* and *Spisula solidissima* and the neurohemoglobin-less glial cells (arrowheads) of *Tagelus plebeius* and *Geukensia demissa*.

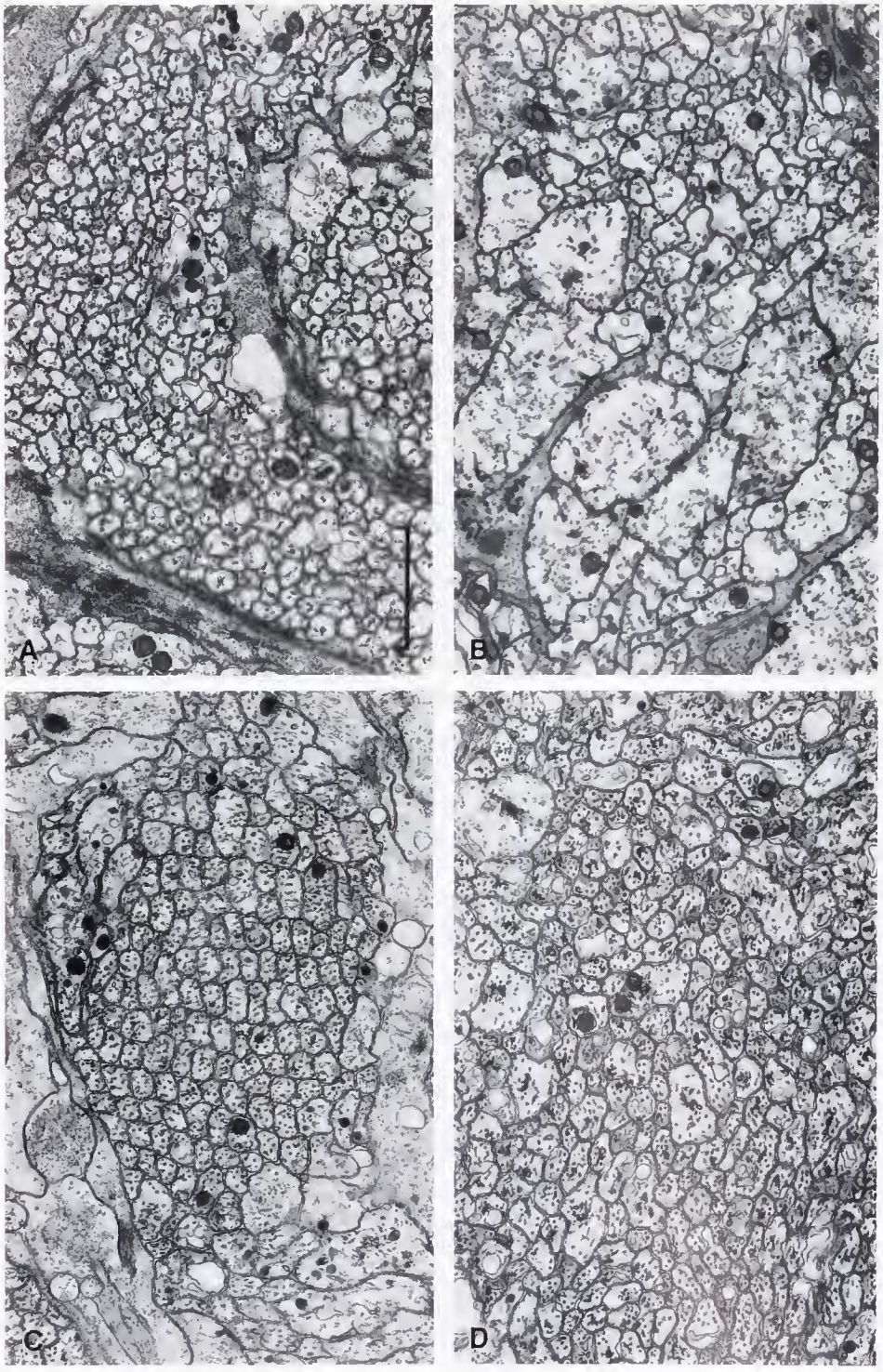

Figure 4. Axons of bivalve cerebro-visceral connectives. A. *Tellina alternata* B. *Spisula solidissima* Note the presence of several larger diameter axons. See Figure 6 for axon size histograms. C. *Tagelus plebeius.* D. *Geukensia demissa.* Bar is 1 μm for A–D. The tightly packed axons in all connectives leave little extracellular space.

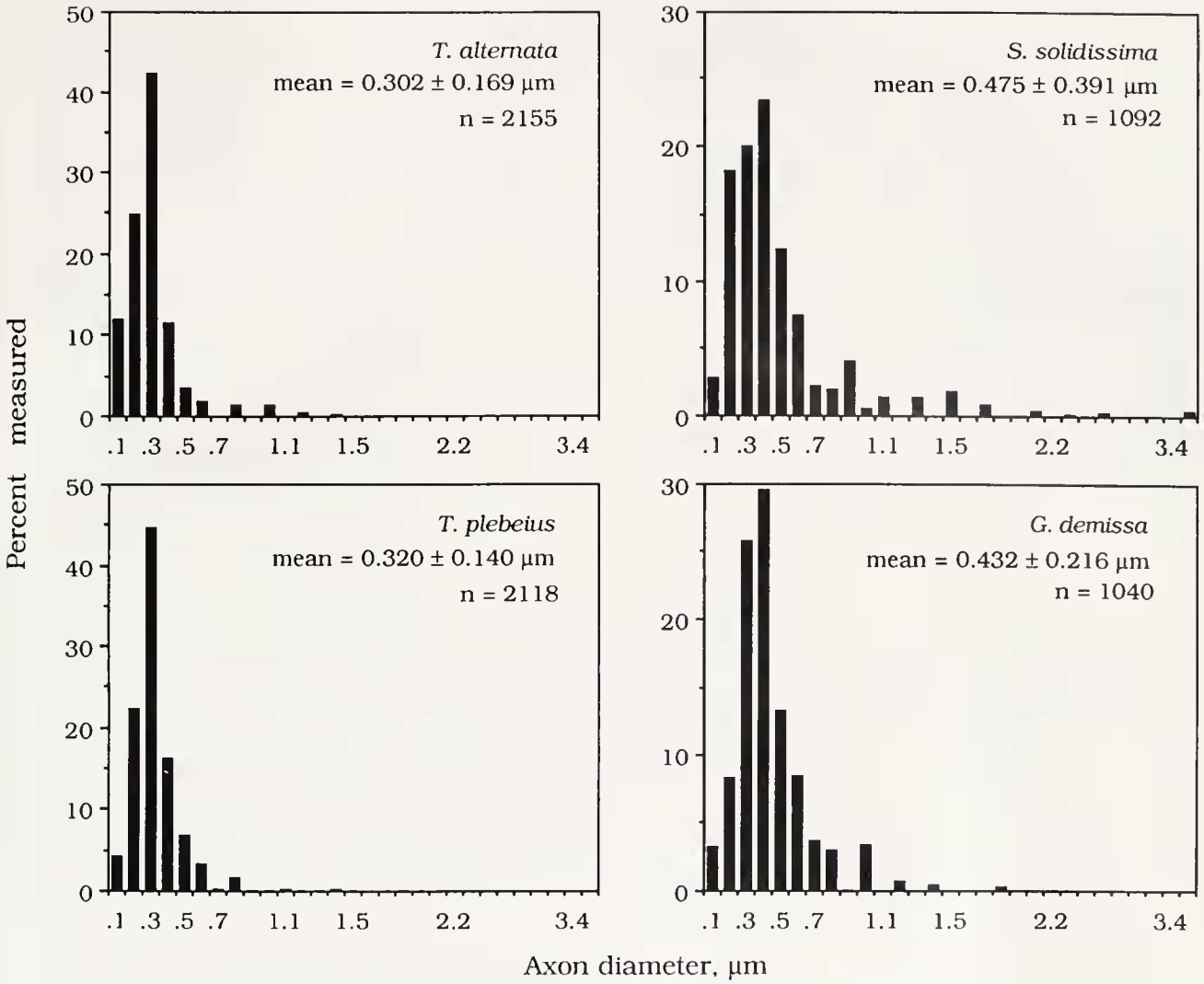

Figure 5. Axon size distributions of bivalve cerebro-visceral connectives. The mean axon diameter (± standard deviation) is given for each species, n is the number of axons measured. Axon diameters were sorted into 0.1 µm divisions for axons 0.1–0.8 µm diameter and 0.2 µm divisions for axons above 0.8 µm diameter. Axons oblong in cross-section were assigned an average diameter based on their long and short diameter.

slow, large-amplitude biphasic wave (Fig. 6). The slow, large-amplitude wave presumably represented action potentials conducted by the majority of small diameter (0.2–0.5 µm) axons. The faster low-amplitude waves preceding the major spike presumably represented action potentials from a small group of fibers greater than 0.5 µm in diameter. A small number but significant volume of the axons from *S. solidissima* connectives are 1.5–3.5 µm diameter (Figs. 4, 5) and this is reflected in the pronounced higher conduction velocity spike (Fig. 6). All electrophysiological measurements were made with the predominant slower velocity spike.

At near-habitat temperatures (10°C for *S. solidissima* and 20°C for the others), the maximum stimulus voltage of all connectives for total recruitment was 2–4 V at 1 ms duration. Externally recorded spike amplitudes at maximum stimuli measured 2–8 mV. Willmer (1978b) reported a 6–10 V spike amplitude in *Mytilus edulis* cerebro-visceral connectives, measured with sucrose gap. Spike duration lasted 15–30 ms at a conduction distance of about 10 mm. Generally, the peak amplitude decreased by about 50% per centimeter length of connective conducted, presumably as the separate components of the compound action potentials became temporally dispersed.

Conduction velocities were measured at peak action potential amplitude over conduction distances of 4–20 mm between the stimulating and recording electrodes

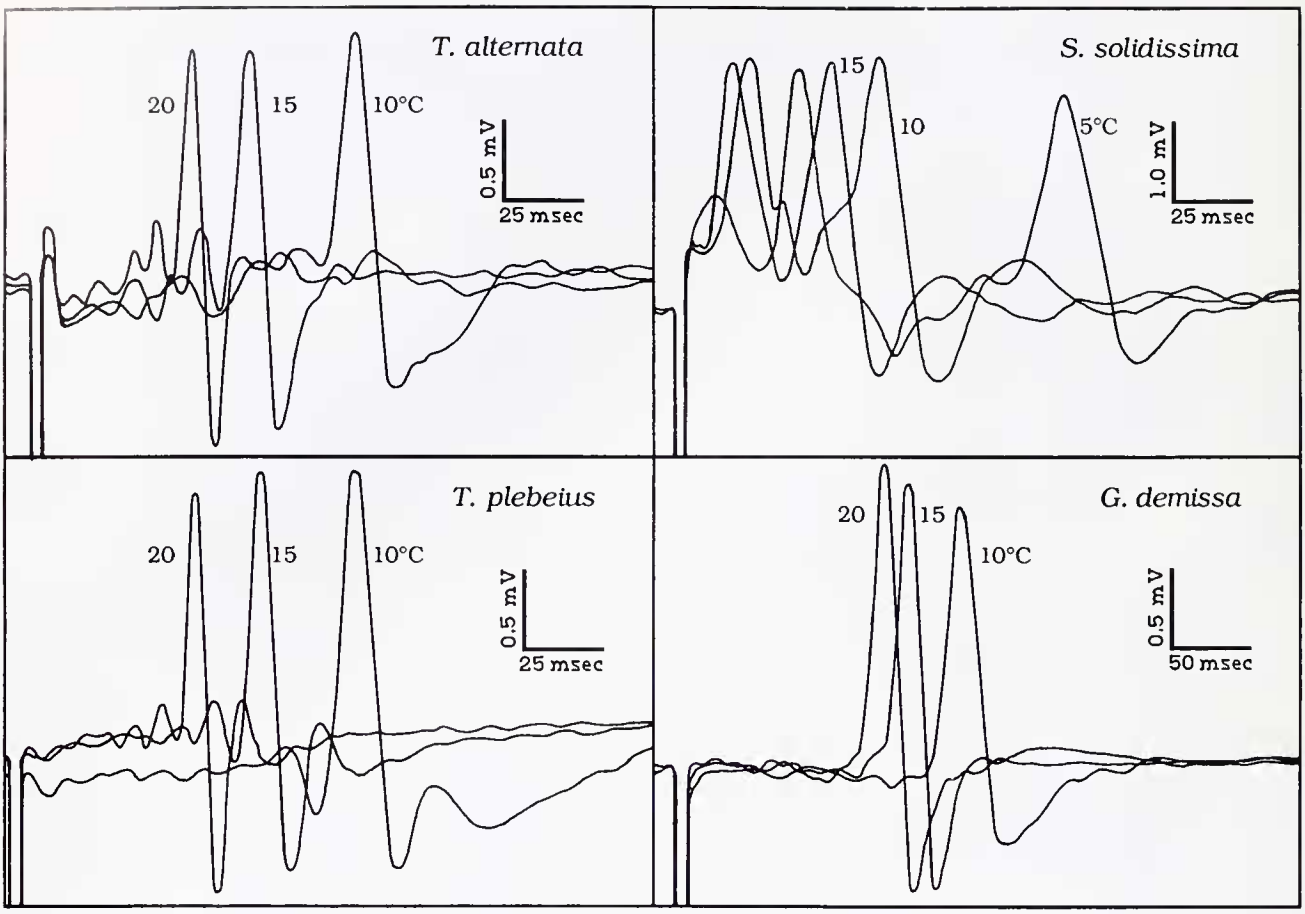

Figure 6. Externally recorded action potential traces of bivalve cerebro-visceral connectives at different temperatures. The stimulus artifact occurs on the left of each trace.

(Fig. 7). Conduction velocities corresponding to near-habitat temperatures were comparable: 21.7 ± 2.9 cm/s for *T. alternata*, 19.5 ± 3.4 cm/s for *S. solidissima*, 18.9 ± 2.4 cm/s for *T. plebeius*, and 14.7 ± 3.1 cm/s for *G. demissa*. Differences could not be attributed to the presence of neurohemoglobin ($P > 0.5$). There was no correlation between conduction velocities and conduction distances at all temperatures, indicating relatively constant conduction velocities along entire connective segments. Horridge (1958) reported 20–30 cm/s as the conduction velocity of cerebro-visceral connectives from the bivalve *Mya arenaria*. Other nerves with small diameter unmyelinated axons have similar conduction velocities: 19.4 cm/s for the garfish olfactory nerve (0.24 μm diameter axons; Ritchie and Straub, 1975) and 14.0 cm/s for the medial bundle-lateral portion of the burbot olfactory nerve (0.1–0.5 μm diameter axons; Doving and Gemne, 1965).

Increases in conduction velocity due to temperature increase were relatively similar among the connectives studied (Fig. 7). The computed temperature coefficients (Q_{10}s) showed little variation between connectives with and without neurohemoglobin. Conduction velocity Q_{10}s for other nerves with small axons are: 1.9, between 8 and 22°C, for garfish olfactory nerve (Ritchie and Staub, 1975), and 1.4, between 8 and 34°C, for burbot olfactory nerve (Doving and Gemne, 1965). Other similar Q_{10}s are 1.7, between 13 and 23°C, for giant fibers from the earthworm (Lagerspetz and Talo, 1967), and 2.2, between 5 and 30°C, for vertebrate myelinated A-fibers (Gasser, 1931).

Refractory periods corresponding to near-habitat temperatures of each species also have similar values (Fig. 8). The average absolute refractory periods are: 33.84 ± 19.36 ms for *T. alternata*, 36.65 ± 16.00 ms for *S. solidissima*, 27.04 ± 2.89 ms for *T. plebeius*, and 47.59 ± 11.56 ms for *G. demissa*. Again, the differences are not associated with the presence of neurohemoglobin ($P > 0.5$). The small axon olfactory nerves from fish exhibit similar absolute refractory periods: 28 ms for pike (Gasser, 1956) and 16 ms for burbot (Doving and Gemne, 1965). Although Q_{10}s were variable among the species

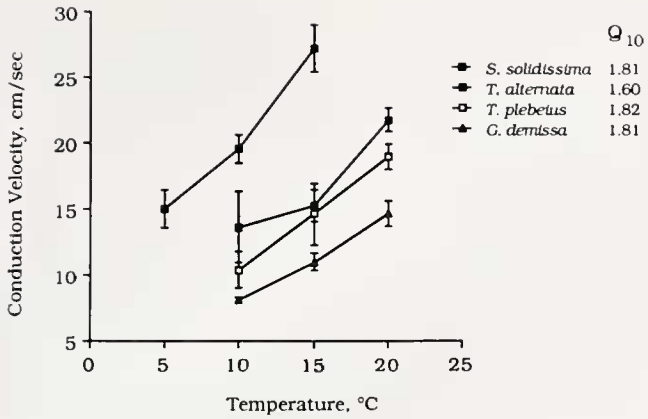

Figure 7. Conduction velocities of bivalve cerebro-visceral connectives as a function of temperature. Q_{10} values were computed over entire temperature range for each species. Data points represent the mean of 20–40 measurements (± standard error).

tested, there were no consistent differences between connectives with and without neurohemoglobin. The Q_{10}s for the absolute refractory period of vertebrate myelinated A-fibers average 5.75, between 5 and 30°C (Gasser, 1931).

Examples of threshold curves at near-habitat temperatures for the four species are presented in Figure 9. The lower stimulus strengths at each duration represent the threshold voltages required for initiating the first axons to fire and the upper values represent the maximum voltages required for recruitment of all axons. Again, consistent differences were not detectable between connectives with and without neurohemoglobin. As temperature was lowered, the curves shifted to the right as the axons became less sensitive. The changes in stimulus threshold in response to temperature were more pronounced at short duration.

Discussion

In general, the cerebro-visceral connectives with and without neurohemoglobin from the bivalve molluscs of this study are anatomically similar to each other and to homologous connectives from other bivalves (Gupta *et al.*, 1969; Willmer, 1978b). In addition, axon density, axon size distribution, and axonal membrane area per gram fresh tissue weight are similar to each other (Table II; Fig. 5) and to the unmyelinated garfish olfactory nerve, a neural preparation valued for its use in studying properties of axon membranes (Easton, 1971). The bivalve cerebro-visceral connective acts as the main communication link between the cerebropleural and visceroparietal ganglia. Consequently, the connective is free for the most part from specialized sensory and large diameter motor fibers and ganglion cells, and consists mainly of unspecialized axons (Horridge and Bullock, 1965). The garfish olfactory nerve participates mainly in relaying information from the olfactory receptors to the olfactory bulb and therefore is also composed of small unspecialized axons. In contrast, the rabbit cervical vagus (Keynes and Ritchie, 1965) and the walking leg nerves from the spider crab (Abbott *et al.*, 1958) are both multifunctional nerves and possess a broad distribution of axon sizes.

The electrophysiological characteristics of bivalve cerebro-visceral connectives are governed mainly by the axon size distribution and mean axon diameter, not by the presence or absence of neurohemoglobin. Compound action potentials are relatively simple (Fig. 6), reflecting the homogeneity of fiber size. The homogeneous fiber spectra of the pike and garfish olfactory nerves result in action potentials of similar quality (Gasser, 1956;

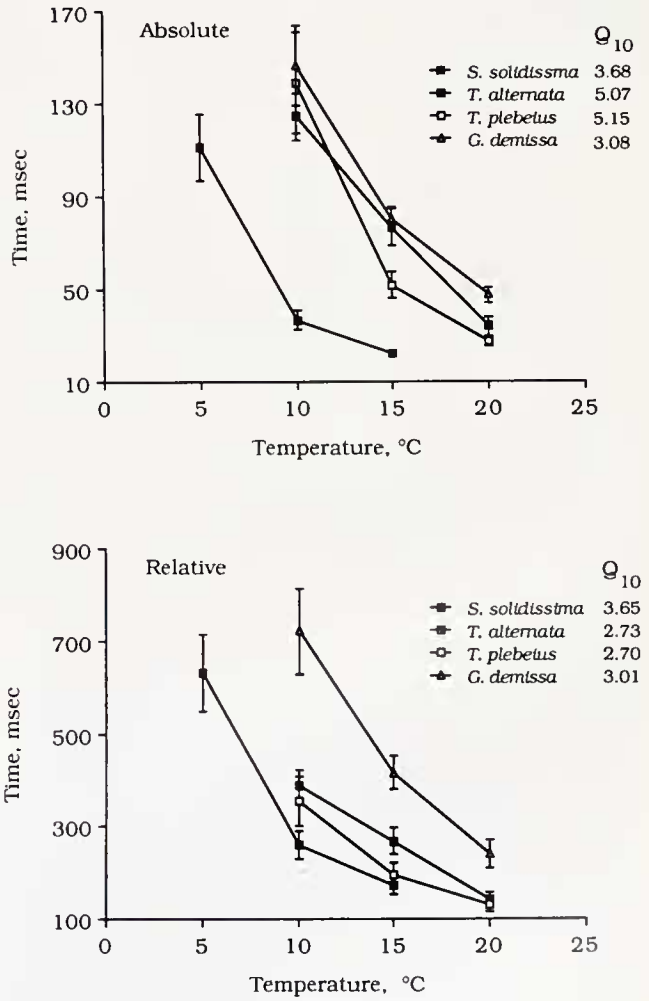

Figure 8. Refractory periods of bivalve cerebro-visceral connectives as a function of temperature. Q_{10} values were computed over entire temperature range for each species. Data points represent the mean of 15–20 measurements (± standard error).

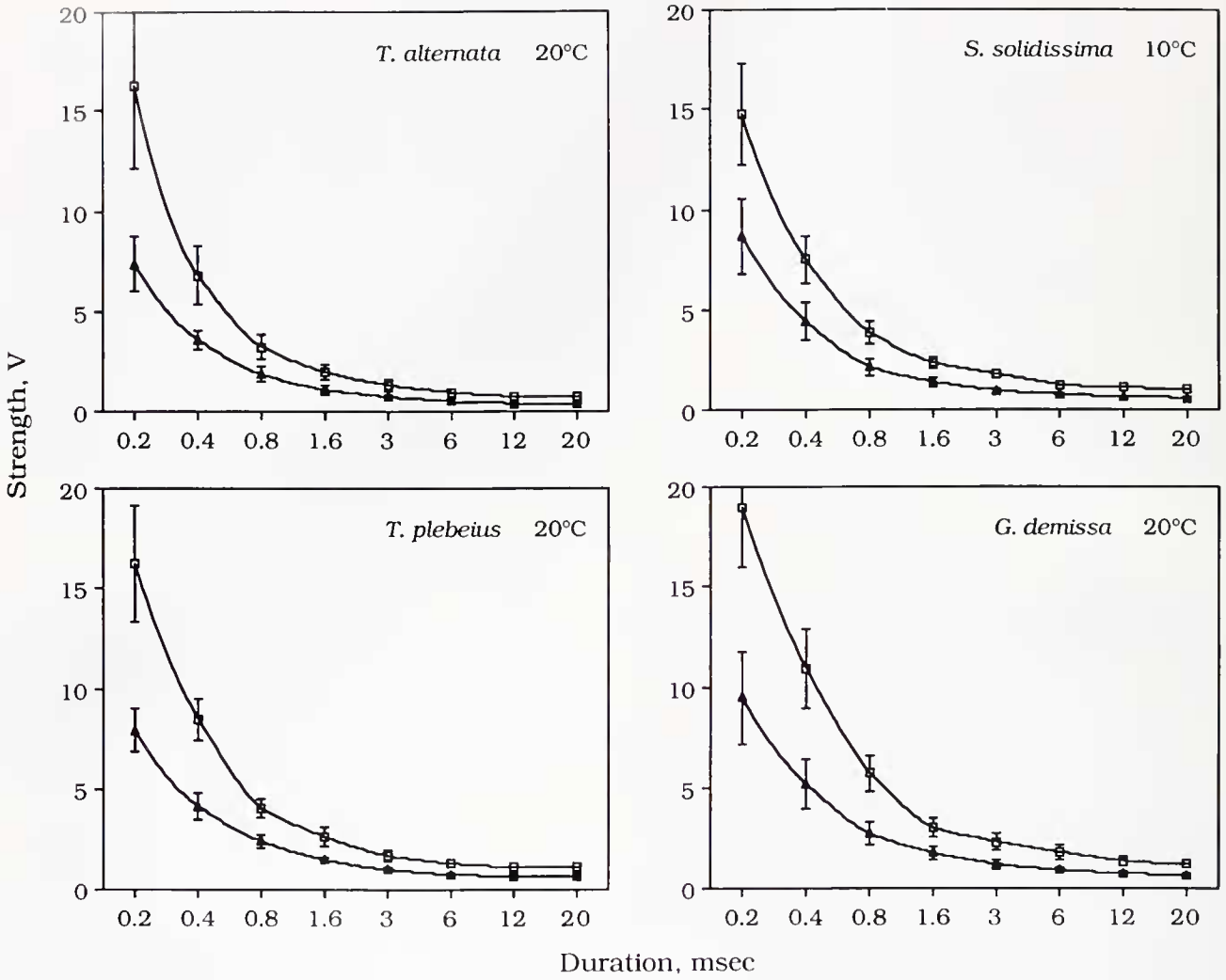

Figure 9. Strength-duration curves for bivalve cerebro-visceral connectives at near-habitat temperatures. Upper curves represent maximum recruitment of axons, lower curves represent threshold recruitment of axons. Each abscissa is scaled as a pseudo-geometric progression in order to best present the data. Data points represent the mean of 3–5 measurements (± standard error).

Ritchie and Straub, 1975). Gasser (1956) noted that this simplicity is not due merely to short conduction distances (4–20 mm in this study) because compound action potentials from the frog sciatic nerve display a complex series of peaks after 7 mm of conduction. Rather, it is due to the relatively narrow range of fiber sizes.

The small difference between the threshold stimulus voltage required for initial recruitment of axons and that required for maximum recruitment (Fig. 9) again demonstrates the constancy of axon type in cerebro-visceral connectives. Low conduction velocities indicate that axon caliber is very small (Fig. 7). Similar conduction velocities and other action potential characteristics recorded in both directions reflect the bidirectional functionality of cerebro-visceral connectives. Horridge (1958) mapped bivalve neural pathways in the whole organism and found that cerebro-visceral connectives function in bidirectional communication. These features are typical of all unmyelinated nerves with small diameter axons reported thus far (see Results for details).

Glial cells support axon function in a variety of ways such as nutrition and ion regulation (Somjen, 1975; Orkland, 1982). In bivalve cerebro-visceral connectives in which glial cells have been modified to produce hemoglobin, axon support, which may include an oxygen supply, may be more extensive than in connectives without neurohemoglobin. Based on the parameters measured under normoxic conditions at low frequency stimulation and at different temperatures, the neurohemoglobin-containing glial cells do not appear to alter action poten-

tial conduction from that exhibited by connectives without neurohemoglobin. However, glial cell participation in action potential conduction may only become evident under more stressful conditions such as low oxygen or high frequency stimulation. This is the subject of a subsequent paper (Kraus and Doeller, submitted).

In contrast to the similarity in electrophysiological characteristics described above, Kraus and Colacino (1986) reported a striking functional difference between the cerebro-visceral connectives of *T. alternata* with neurohemoglobin and *T. plebeius* without neurohemoglobin. Under anoxic conditions, the electrical excitability of connectives with neurohemoglobin lasted 20–30 minutes whereas the electrical activity of connectives without neurohemoglobin lasted only about 5 minutes (Kraus and Colacino, 1986). This functional difference (which has also been observed between the cerebro-visceral connectives of *S. solidissima* and *G. demissa:* Kraus and Doeller, submitted) may result in part from differences in the ultrastructural design of connectives with and without neurohemoglobin.

The acquisition of neurohemoglobin by glial cells to support axon function may have occurred commensurately with changes in ultrastructure that allowed maximum use of this oxygen supply. For example, smaller axon bundles caused by increased ramification of glial cell processes would have smaller mean diffusion distances between axon and glial cell. This would increase the probability that oxygen molecules released from the glia would reach the axons rather than diffuse out of the nerve. Smaller axon bundles and smaller diffusion distances are characteristic of the two cerebro-visceral connectives with neurohemoglobin (Table II). In addition, an oxygen diffusion barrier between the neurohemoglobin and the outside would tend to keep dissociated oxygen molecules within the connectives during anoxic conditions and would lengthen the time that oxygen is available for aerobic metabolism. The highly organized perineural sheath with its densely staining multilaminar structure, characteristic of the two connectives with neurohemoglobin (Fig. 2), may represent a diffusion barrier to oxygen.

To summarize, bivalve cerebro-visceral connectives with and without neurohemoglobin exhibit no significant differences in electrophysiology under normoxic conditions. Action potential conduction is influenced mainly by mean axon diameter. On the other hand, differences in ultrastructure between the two types of connectives may be related to the presence of neurohemoglobin, and oxygen delivery from glial cells to axons in neurohemoglobin-containing connectives may be aided by smaller diffusion distances and a dense perineural sheath. To understand why one type of nervous system has glial cells with neurohemoglobin and the other does not, it will be necessary to determine the functional characteristics of the neurohemoglobin, the oxygen demand of each type of cerebro-visceral connective, and the electrophysiological behavior of the connectives exposed to periods of anoxia. These will be the subjects of subsequent papers (Doeller and Kraus, 1988; Kraus and Doeller, submitted).

Acknowledgments

This work represents part of a Ph.D. dissertation submitted by D.W.K. to Clemson University. We thank Dr. J. M. Colacino for help and advice throughout the project, Dr. E. E. Ruppert, T. L. Vandergon, and J. M. Turbeville for helpful discussions of the work, and Dr. J. B. Wittenberg for critical review of this manuscript. We thank E. Wagner at Rutgers Shellfish Research Laboratory and R. Goldberg at NOAA for the supply of *Spisula solidissima.* This work was supported in part by grants from Sigma Xi, the Slocum-Lunz Foundation, and the Lerner-Grey Fund for Marine Research of the American Museum of Natural History.

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